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Antagonistic Activity of Some Bioagents against Root Rot Diseases of Pepper (*Capsicum annum* L.)

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EIGHT isolates of bioagents (fungal and bacterial), obtained from the rhizosphere of healthy pepper plants collected from various pepper-growing sites in Kafr El-Sheikh governorate, were tested against pepper root-rot pathogens including *Pythium aphanidermatum, Fusarium solani, F. oxysporum, F. moniliformis* and *Macrophomina phaseolina*. In i*n vitro* study, the fungal bioagents (*Trichoderma viride* (TV1 and TV2) and *T. harzianum* (TH1 and TH2) exhibited the maximum antifungal activity against the five phytopathogens compared to the bacterial bioagents (*Pseudomonas fluorescens* (P1 and P2) and *Bacillus subtilis* (B1 and B2). In pots experiment, TV1 and P2 bioagents caused the least disease severity among all treatments. All bioagents were effective with different degrees specially *Trichoderma* spp. to promote the growth parameters of pepper plants and manage root rot disease that caused by different pathogens. Furthermore, they had equal efficacy with fungicide treatment under *in vitro* and in pots experiment. Thus, application of biological methods in plant disease control is an effective alternative technique and could have a potential biofertilizer effect, since they stimulated the growth of pepper plants.

Keywords: Pepper, Biological control, Root rot, Bioagents

Introduction

Sweet pepper (*Capsicum annuum* L.) belongs to the family Solanaceae, which is an important group of vegetables extensively cultivated in Egypt and all over the world. The total cultivated area of pepper in Egypt reached 41,047 ha and producing 623,221 tons annually (FAO, 2017). Pepper plants are liable to be attacked by several soil-borne pathogenic fungi, which are responsible for a considerable plant mortality and consequently high losses in the yield and quality in many countries of the world (Van Bruggen and Semenov 2000). *Fusarium solani*, *F. oxysporum*, *Pythium aphanidermatum* and *Macrophomina phaseolina* cause root rot diseases in sweet pepper plants (Kelley *et al*., 2009; Wilson *et al*., 2010 and Mmbaga & Gurung, 2018).

Biological control is a viable strategy for disease management and sustainable pepper production (Velivelli et al., 2014). Bioagents antagonize pathogens directly by hyperparasitism, production of antibiotics and lytic enzymes, and indirectly by competing for space and nutrients, inducing systemic resistance, and promoting plant growth (Pal and Gardener, 2006; Velivelli et al., 2014). Bioagents secrete enzymes such as chitin, proteins, cellulose, and hemicellulose that are able to hydrolyze mycelium and spores of fungal pathogen as a direct suppression of plant pathogens (Solanki et al., 2011).

Trichoderma spp. is one of the important bioagents used for management of different diseases in several crop plants (Kubicek et al., 2001; Hussain et al., 2017; Omara et al., 2017). Genus *Trichoderma* as an active soil inhabitant and root system colonizers possibly as plant symbionts as well as a parasitic to some pathogenic fungi were described by Harman et al. (2004). *T. viride* was reported to have the ability

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to inhibit soil borne pathogenic fungi *in vitro*. The average of inhibitory coefficient against *F. oxysporum*, *F. solani* and *R. solani* was between 70 to 80% and was attributed to either toxin production or mycoparasitism (Howell, 2003).

Pseudomonas fluorescens and *Bacillus subtilis* as bioagents were reported to suppress certain fungi causing root rot diseases in sweet pepper plants by producing a variety of microbial metabolites like siderophores and production of extracellular cell wall degrading enzymes, as chitinase that can lyse pathogen cell walls or plant growth regulators as IAA (Yu et al., 2011). The cell wall degrading enzymes from *P. fluorescens* has a great potential in agriculture as active components in new fungicidal formulation as found by El-Gamal et al. (2016).

The main objective of this study was to evaluate the efficacy of *Trichoderma harzianum*, *T. viride*, *Pseudomonas flouresence* and *Bacillus subtillus* in controlling pepper root rot disease *in vitro* and under greenhouse conditions compared with an effective fungicide.

Materials and Methods

Isolation, purification and identification of pathogenic fungi

Pepper plants showing root rot symptoms were collected from Kafr El-Sheikh governorate during all cultivation seasons of pepper plants in 2016/2017. Diseased roots were washed with tap water, cut into small pieces and surface sterilized by 0.5% sodium hypochlorite solution for three minutes and then, washed three times with sterilized distilled water. Samples were dried between two layers of sterilized filter papers to remove the excess water and placed on potato dextrose agar (PDA) medium in Petri dishes. Inoculated dishes were incubated at 18-20 °C for 4-5 days, and the developed fungal cultures were purified by using hyphal tip isolation techniques (Brown, 1924). They were identified based on the morphological and microscopic characteristics. Identification was confirmed in the Mycological Research and Disease Survey Department, Plant Pathology Research Institute (PPRI), ARC, Giza, Egypt. The pathogenic isolates were maintained on PDA at 5±1 °C.

Pathogenicity test

Pathogenicity test was conducted in October 2017/2018. The identified isolates (*P. aphanidermatum, F. solani, F. oxysporum, F. moniliformis* and *M. phaseolina*) were grown on barley-sandy medium (140 g barley grains, 60 g sand and 60 ml water) for two weeks (Abdel-Monaim and Ismail, 2010) at 25±1°C to test their pathogenicity. Plastic pots (30 cm in diameter) containing 5kg/pot sterilized sandy-clay (1:1 w/w) were infested with prepared inoculum at 5.0%, for ten days before transplanting. Healthy seedlings (30 days old) of pepper cv. Top star were transplanted at the rate of 5 seedlings/pot. Five pots were used for each fungus. Check treatment (control) was used. The pots were kept under careful observation under greenhouse condition of the Plant Pathology Dept., Sakha station, Kafr El-Sheikh governorate, Egypt.

Disease assessment

Disease severity index and disease incidence were determined on the foliar part and vascular tissue discoloration after 45 days from transplanting. Pepper plants were removed from the soil, washed thoroughly to remove soil debris, and root rot disease severity was scored based on the modified scale of (Hwang and Chang, 1989) as follows:

0= neither root discoloration nor leaf yellowing, 1= 1-25% root discoloration or one leaf yellowed, 2= 26-50% root discoloration or more than one leaf yellowed, 3= 51-75% root discoloration or vascular discoloration plus one leaf wilted, 4= up to 76% root discoloration or more than one leaf wilted or completely dead plants. For each replicate, a disease severity index (DSI) similar to the one described by Liu *et al.* (1995) was calculated as follows:

$$DSI = \Sigma d / (d\ max \times n) \times 100$$

Whereas d is the disease rating of each plant, d max is the maximum disease rating and n is the total number of plants examined in each replicate.

Additionally, the disease incidence was estimated using the following equation:

$$\%\ \text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} 100\times$$

Isolation and identification of antagonistic rhizosphere microorganisms from healthy roots of pepper plants

The serial dilution method was used for isolation of antagonistic rhizosphere microorganisms of healthy pepper roots collected from Kafr El-Sheikh governorates (Iqbal and Ashraf, 2017). After an incubation period, fungal or bacterial colonies were purified and identified according to their cultural, morphological and physiological characters. Bacterial isolates were identified based on Bergey's Manual of Systematic Bacteriology (1984) using the methods recommended by Parry et al. (1983). On the other hand, fungal isolates were subjected to identification tests according to the methods stated by Domsch et al. (1980). Identification was confirmed through both of the Mycological Research and Disease Survey Department and Bacterial Disease Department, Plant Pathology Research Institute, ARC, Giza, Egypt as shown in Table 1.

TABLE 1. Fungal and bacterial isolates used as bioagents

Name of isolate	Code
Trichoderma harzianum 1	TH1
Trichoderma harzianum 2	TH2
Trichoderma viride 1	TV1
Trichoderma viride 2	TV2
Pseudomonas fluorescens 1	P1
Pseudomonas fluorescens 2	P2
Bacillus subtilis 1	B1
Bacillus subtilis 2	B2

Bioagents culture and inoculum preparation

T. harzianum and *T. viride* strains were grown on PDA and incubated at 25±2°C for 7 days and maintained refrigerated at 4ºC until use. *B. subtilis* strains were maintained on nutrient medium (Difco, 1985) for 48h at 28±2ºC and maintained refrigerated at 4ºC until use. Whereas, *P. fluorescens* was cultured and maintained on King's medium (King et al., 1954) at 28±2ºC for 5 days and maintained refrigerated at 4ºC until use.

Antifungal activity of isolated bioagents in vitro

An *in vitro* preliminary antifungal assay was performed using eight bioagents and fungicide Hatric 6% (RS)- 1 -p-chlorophenyl 4,4-dimethyl -3-(1 H- 1,2,4-triazol - 1 -methyl)pentan-3-ol) used as a recommended dose at the rate of (1cm/L water) as a positive control on PDA (for fungi) or nutrient agar (NA) for bacteria using the linear growth method against of *P. aphanidermatum, F. solani, F. oxysporum, F. moniliformis and M. phaseolina*.

Assays were performed in Petri dishes (90 mm) containing 20 ml of PDA or NA medium that were inoculated with a 5 mm disc in diameter of pathogenic isolates at 1cm distance from the edge of Petri dishes, whereas the opposite side (1cm distance from the edge) was inoculated with either discs of *T. harzianum* or *T. viride* isolates or with streaking the *B. subtilis* or *P. fluorescens* strains.

Meanwhile, fungicide treatment was added as recommended dose 1cm/L to the sterilized PDA medium before solidifying and gently rotating and disbanding into sterilized Petri plates and inoculated at the middle with equal disks of pathogenic isolates then incubated at 25±2°C. The antifungal activity of bioagents and fungicide were evaluated as a percentage of inhibition in the mycelial growth of as described by Ferreira et al. (1991) using the following formula:

Where:

% I = Percentage of growth inhibition.

A = the distance of mycelial growth of the pathogenic fungus (control).

B = the distance of mycelial growth of the pathogenic fungus in the treatment.

Greenhouse experiments

Inoculum of pathogenic isolates and soil treatment

Greenhouse experiment was conducted in February 2018/2019 to evaluate the effect of selected bioagents compared with the fungicide on the incidence of root rot disease on pepper plants. The five isolates of root rot fungi mentioned above were grown on barley-sandy medium on glass bottles of 500 ml capacity for two weeks at 25±1°C. Plastic pots (30 cm in diameter) containing sterilized sandy-clay soil (1:1 w/w) were infested individually with inoculums of each fungus. Fungal inoculum was mixed with the sterilized soil surface of each pot at rate of 5% (w/w) potential inoculum. The soil was then moistened with water for ten days before transplanting. Check treatment (control) was prepared without the addition of the tested fungi.

Preparation of bioagents inoculum

Depending on laboratory experiments, the most effective bioagents were selected for further study. Inoculum of the selected bioagents (TV1, TH2, B1 and P2 isolates) in comparison with Hatric 6% fungicide for controlling pepper root rot disease were tested under greenhouse conditions. The experiment was carried out in randomized complete block design (RCBD) with five replicates for each particular treatment.

TV1 and TH2 strains were grown on Potato dextrose broth medium and incubated at 28±2°C for ten days. Spore suspensions of both strains were counted and adjusted at (1×10^6spore/ml) each using a haemocytometer slide. Furthermore, B1 and P2 isolates were separately grown on nutrient broth medium and incubated at 28±2°C for 4-5 days in 250 ml flasks. The density of bacterial cell culture was adjusted at (1×10^8cfu/ml) using a haemocytometer slide (Janisiewicz and Marchi, 1992).

Plant materials

Pepper cv. Top star seeds were obtained from the Department of Horticulture Research Institute, Giza, Egypt. The seeds were grown in the seed beds as nurseries for 4 weeks. In the greenhouse, seedlings roots (30-days old) were surface sterilized by immersing in 0.05% household bleach (Clorox 3.8% NaCl) for 3 min and then washed three times with sterilized water just before treating.

Treatments

The bioagents and the fungicide Hatric 6% were applied by dipping pepper seedling (30 days old) in previous prepared treatments as mentioned before for 30 minutes in fungicide and 2 hours in suspensions of bioagents then seedlings were dried on open air before transplanting in each treatment individually. Then, soil was drenched after 7 days of transplanting with the same previous treatments at the same concentrations with 10 ml of different treatments to each seedling in the pots. Untreated seedling was dipped in sterilized water for 30 minutes and air dried before transplanting as uninfected control. On the other hand, there was just infected control as appositive control according to each treatment individually for each pathogen separately.

Three seedlings were transplanted in each pot and 5 pots as replicates for each treatment. The pots were kept in the greenhouse (25±3°C) and received the recommended dose of N, P and K fertilizers. Irrigation was carried out regularly and the cultivation period was extended up to 45 days after transplanting.

The pots were kept under careful observation under greenhouse condition of the Plant Pathology Dept., Sakha Station, Kafr El-Sheikh Governorate, Egypt. One week after sowing, disease severity was examined then recorded at 15, 30 and 45 days, respectively.

Effect of treatments on growth parameters of pepper plants

Growth parameters of pepper plants including shoot and root length (cm) and dry weight (g) were recorded in different treatments and control after 45 days of transplanting. The roots and shoots of the plant were separated and air dried before dried at 70^0C for 48h in oven till weight stability before getting the dry weight. The experiment was repeated with five replicates of each treatment.

Statistical analysis

Data were analyzed through one way analysis of variance (ANOVA) with the least significant difference (LSD) at the 0.05 probability level and Duncan's Multiple range test using CoStat Version 6.4 (CoHort Software, Monterey, CA).

Results and Discussion

Response of pepper plants to infection by some root-rot pathogens under greenhouse conditions (pathogenicity test)

In the present study, all isolates exhibited significant differences for the tested cultivar Top Star. Furthermore, observation of the symptoms showed significant differences between the isolated fungi for their pathogenicity to pepper plants.

Results in Table 2 shows that the most virulence isolates are *P. aphanidermatum* and *F. solani* that caused the highest disease severity (97.33 % and 95.00%, respectively) and the highest disease incidence (72% and 60%, respectively). On the other hand, the lowest disease severity was achieved with *F. moniliformis* and *M. phaseolina* (80.42 % and 80.63 %, respectively) and they also have the lowest disease incidence (44% and 52%, respectively). These results are in accordance with those reported by many researchers (Jayaraj et al., 2005; Jeyaseelan et al., 2012; Kipngeno et al., 2015; Elshahawy et al., 2018).

In vitro antagonistic effect of bioagents against root-rot pathogens

Different bioagents exhibited different levels of bioactivity against different root-rot pathogens as presented in Table 3. Results revealed that all fungal bioagents as well as the fungicide Hatric showed a degree of inhibition against the mycelial growth patterns of all root-rot pathogens. Contrarily, all bacterial bioagents showed antifungal activity against all tested pathogens except for *P. aphanidermatum*. In addition, *P. fluorescens* isolate 1 (P1) had no effect on *M. phaseolina.*

In general, it is obvious that TH2 reduced the mycelial growth of the five pathogenic fungi at the range of 62.22 to 78.09 %, while the range of the inhibition of the mycelial growth of the same pathogens by TV1 was from 68.89 to 87.53%. Results also indicated that B1 isolate reduced the mycelial growth of the four pathogenic fungi by 29.87 to 44.07%, while P2 isolate reduced the mycelia growth by 33.20 to 43.67% (Table 3 and Fig. 1-5).

It is clear that the fungal bioagents significantly reduced the mycelial growth of the tested pathogenic fungi higher than the bacterial agents did. Our results are in an agreement with those recorded by Karima and Nadia (2012), who reported that *Trichodermia* spp. had an antagonistic ability and decreased the mycelial growth of *R. solani* but also have antifungal effect against *Botrytis cinerea, Fusarium oxysporium, Macrophomina phaseolina* and *R. solani* (Talla *et al.,* 2015). Additionally, *B. subtilis* and *T. harzianum* were

TABLE 2. Response of pepper plants to infection by some root-rot pathogens under greenhouse conditions

Pathogens	%Disease incidence (DI%)	%Disease severity (DS%)	
		Foliar part	Vascular tissue
Pythium aphanidermatum	72	80.51a	97.33a
Fusarium solani	60	75.60b	95.00a
Fusarium oxysporum	52	70.10c	87.50b
Fusarium moniliformis	44	60.50d	80.42c
Macrophomina phaseolina	52	60.81d	80.63c
Control	0	0.00e	0.00d
L.S.D. at 0.05	-	4.05	4.43

- Mean values in each column followed by the same letter are not significantly different (P ≤0.05).

TABLE 3. *In vitro* antagonistic effect (%inhibition) of bioagents against root-rot pathogens

Treatment	Pathogens				
	Pythium aphanidermatum	***Fusarium solani***	***Fusarium oxysporum***	***Fusarium moniliformis***	***Macrophomina phaseolina***
T. harzianum (TH1)	59.30e	70.33c	81.48a	61.83c	62.22c
T. harzianum (TH2)	78.09c	68.00c	69.26c	75.92a	62.22c
T. viride (TV1)	87.53b	80.80a	75.93b	68.89b	76.29b
T. viride (TV2)	65.15c	73.38b	71.85c	67.78b	59.27e
P. fluorescens (P1)	0.00 f	35.93e	34.80f	30.00e	0.00g
P. fluorescens (P2)	0.00f	41.83 d	43.67 e	42.57 d	33.20 f
B. subtilis (B1)	0.00f	44.07 d	43.70 e	42.56 d	29.87 f
B. subtilis (B2)	0.00f	43.67 d	43.67 e	38.90 d	33.20 f
Hatric	93.30a	43.70 d	62.00d	78.52 a	94.40 a
Control	0.00f	0.00 f	0.00g	0.00 f	0.00 g
L.S.D. at 0.05	0.32	2.50	3.81	3.19	2.82

- Mean values in each column followed by the same letter are not significantly different (P ≤ 0.05).

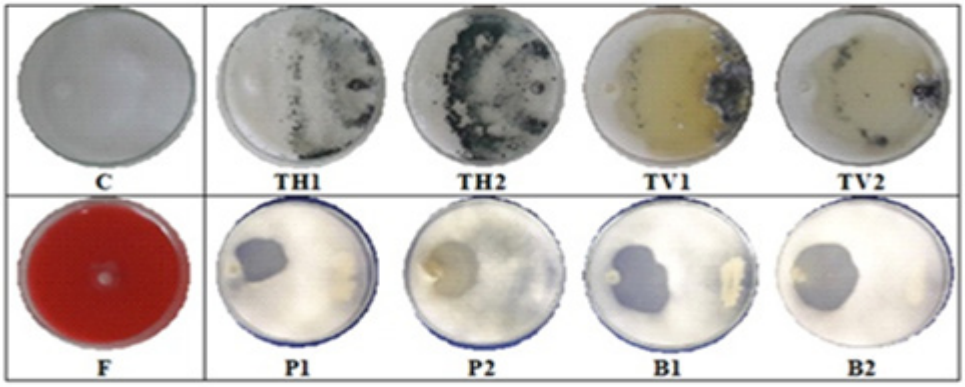

Fig. 1. Inhibition of *Pythium aphanidermatum* (C) growth by bioagents
- TH1, TH2: *Trichoderma harzianum,* TV1, TV2: T. viride, P1, P2: *Pseudomonas flouresence,* B1, B2: *Bacillus subtilis,* F: Hatric fungicid.

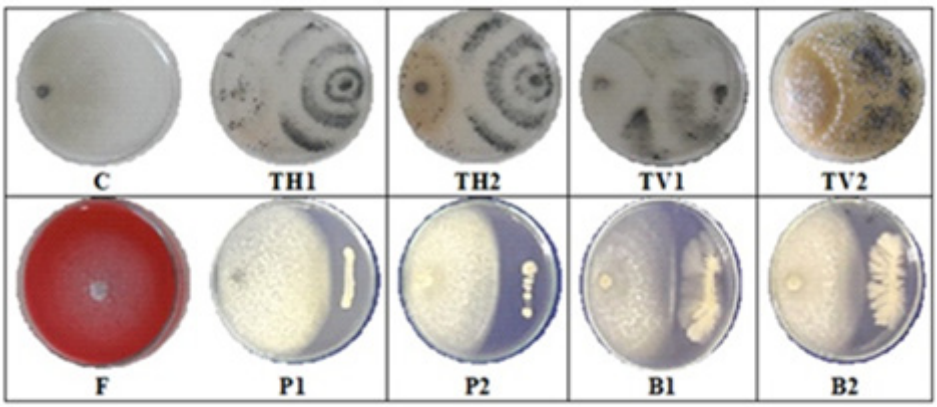

Fig. 2. Inhibition of Fusarium solani (C) growth by bioagents
- TH1, TH2: *Trichoderma harzianum,* TV1, TV2: T. viride, P1, P2: *Pseudomonas flouresence,* B1, B2: *Bacillus subtilis,* F: Hatric fungicid.

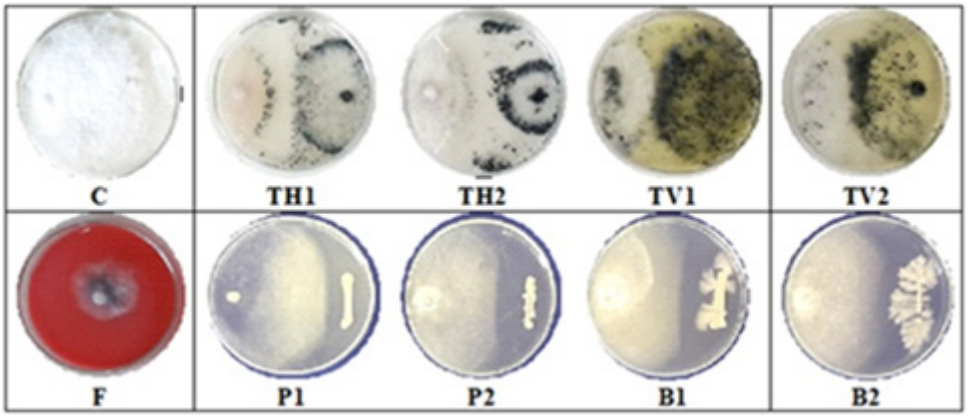

Fig. 3. Inhibition of *Fusarium oxysporum* (C) growth by bioagents
- TH1, TH2: *Trichoderma harzianum,* TV1, TV2: T. viride, P1, P2: *Pseudomonas flouresence,* B1, B2: *Bacillus subtilis,* F: Hatric fungicid.

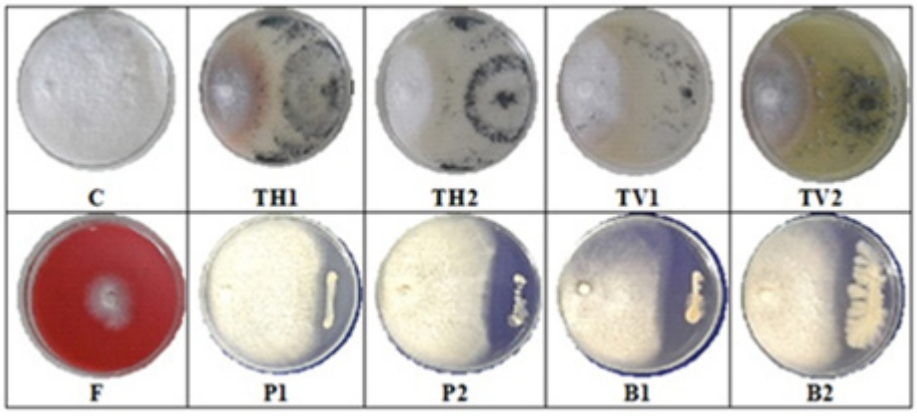

Fig. 4. Inhibition of *Fusarium moniliformis* (C) growth by bioagents
- TH1, TH2: *Trichoderma harzianum,* TV1, TV2: T. viride, P1, P2: *Pseudomonas flouresence,* B1, B2: *Bacillus subtilis,* F: Hatric fungicid.

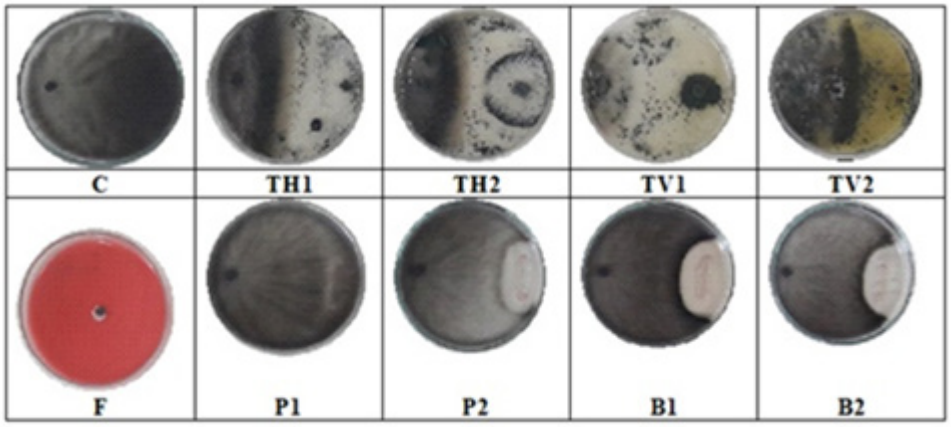

Fig. 5. Inhibition of *Macrophomina phaseolina* (C) growth by bioagents - TH1, TH2: *Trichoderma harzianum,* TV1, TV2: T. viride, P1, P2: *Pseudomonas flouresence,* B1, B2: *Bacillus subtilis,* F: Hatric fungicid.

effective against *M. phaseolina* in *Geranium* sp. (Ghazi et al., 2018). Furthermore, it has been mentioned that the antagonistic effect of Trichoderma may be due to faster mycelia growth than pathogenic fungi (Melo and Foull, 2000; Huang et al., 2011). Meanwhile, *B. subtilis and P. fluorescens* also play an important role in controlling the soil-borne pathogens by producing the antibiotics and sidrophoroes (Roberti and Selmi, 1999).

Effect of some bioagents on pepper root-rot pathogens under greenhouse conditions

From the *in vitro* study, two fungal bioagents *T. viride* (TV1) and *T. harzianum* (TH2) as well as two bacterial isolates *B. subtilis* (B1) and *P. fluorescens* (P2) that were superior to other bioagents, were selected for continues studies under greenhouse conditions. The fungicide Hatric was used as a positive control in this experiment.

Results presented in Table 4 show that the highest antagonistic effect against *P. aphanidermatum* was obtained with the application of P2 isolate and Hatric (without significant differences between them), as they exhibited 66.3 and 66.03% efficacy, respectively. In the case of *F. solani*, the highest percent efficacy was achieved with TH2, TV1 or Hatric fungicid, which recorded 68.4, 66.08 and 68.1% efficacy, respectively. Furthermore, no significant differences were detected between TV1 and Hatric treatments towards *F. oxysporum* with 66.7 and 66.5% efficacy, respectively. For *F. moniliformis*, Hatric possessed the strongest effect recording 66.4% efficacy, followed by B1 and P2 with 41.0 and 40.5% efficacy, respectively. Moreover, Hatric fungicide reduced the disease severity of *M. phaseolina* with a 79.3% efficacy, followed by TV1 and P2 that recoded 67.8 and 67.5% efficacy, respectively.

Strains of *Pseudomonas* have shown efficacy in controlling a number of fungal diseases, including *Fusarium* wilt in cotton and tomato (Gamliel and Katan, 1993) and *R. solani* root infection in tomato (Siddiqui and Shaukat, 2002).

The high effect of *T. viride* and *T. harzianum* could be explained due to the antibiotic substances, which are produced in sufficient concentration to affect the growth of soil fungi *M. phaseolina* (Sreedevi *et al.,* 2011 and Muhanna et al., 2016). Moreover, the beneficial effect of *T. viride* and *T. harzianum* are due to direct mycoparasitism on the pathogenic fungi and to effects on plants such as enhancement plant development and inducing resistance (Harman, 2000).

Effect of bioagents on root and shoot length of pepper plants infected with root-rot pathogens

As shown in Table 5, root and shoot length of pepper plants, noted 45-days after transplanting, depended significantly on the antagonistic treatments tested. Most of the bioagents significantly promoted the length of roots and shoots of pepper plants infected with *P. aphanidermatum, F. solani, F. oxysporum, F. moniliformis* and *M. phaseolina* compared to inoculated or untreated control. Results indicated that *P. aphanidermatum* had the maximum root and shoot length when treated with P2 bioagent (12.5 and 32.0 cm), while TH2 enhanced the root and shoot length of *M. phaseolina* recorded 9.9 and 19.5 cm. Furthermore, TV1 bioagent possessed the highest root and shoot length of *F. solani, F. oxysporum* and *F. moniliformis* isolates, which recorded 11.87 and 26.83 cm, 14.13 and 33.20 cm and 9.77 and 22.37 cm, respectively (Table 5). It has been previously reported that *Trichoderma* are beneficial to several crop plants not only by promoting their growth but also by protecting them from the disease infection through triggering the systematic resistance (Shivanna et al., 1996).

TABLE 4. Effect of some bioagents on pepper root-rot pathogens under greenhouse conditions

Treatment	Pathogens									
	Pythium aphanidermatum		*Fusarium solani*		*Fusarium oxysporum*		*Fusarium moniliformis*		*Macrophomina phaseolina*	
	DS%*	E%*	DS%	E%	DS%	E%	DS%	E%	DS%	E%
T. harzianum (TH2)	74.60 a	0.92	25.3d	68.40	74.50a	1.80	75.5b	11.70	30.5 c	61.50
T. viride (TV1)	32.90 c	56.31	27.3d	66.08	25.3d	66.70	75.8 b	11.40	25.17d	67.80
P. fluorescens (P2)	25.30 d	66.30	75.5b	6.20	30.43c	60.10	50.9 c	40.50	26.03d	67.50
B. subtilis (B1)	50.80 b	32.50	50.6c	32.50	50.2b	33.90	50.5c	41	50.5b	9.80
Hatric	25.55 d	66.03	25.7d	68.10	25.3d	66.50	28.53d	66.40	14.8e	79.30
Infected Control	75.30 a	-	80.5a	-	75.9a	-	85.6a	-	71.5a	-
Untreated Control	0.00 e	-	0.00e	-	0.00 e	-	0.00 e	-	0.00 f	-
L.S.D. at 0.05	1.92	-	2.60	-	3.32	-	2.00	-	3.09	-

- Mean values in each column followed by the same letter are not significantly different (P ≤ 0.05).
- DS%*: Disease Severity percentage.
- E%*: Efficacy percentage.

TABLE 5. Effect of bioagents on root and shoot length of pepper plants infected with root-rot pathogens

Treatment	Pathogens									
	Pythium aphanidermatum		*Fusarium solani*		*Fusarium oxysporum*		*Fusarium moniliformis*		*Macrophomina phaseolina*	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
T. harzianum (TH2)	9.17c	24.67b	9.83b	24.83b	9.00 c	17.20d	6.90c	15.67d	9.90a	19.50a
T. viride (TV1)	8.17d	19.50d	11.87a	26.83a	14.13a	33.20a	9.77a	22.37a	7.77b	15.83d
P. fluorescens (P2)	12.5a	32.00a	8.17c	13.67e	9.03 c	16.23e	7.97b	17.16c	5.83d	11.70f
B. subtilis (B1)	11.43b	22.00c	10.10b	25.16b	9.90b	24.43b	7.73b	14.90e	6.97c	16.77c
Hatric	8.17d	17.56d	6.30d	15.23d	5.36d	13.80f	4.37d	12.63f	4.40e	12.60e
Infected Control	3.07b	14.47e	2.90e	12.80f	3.13e	10.13g	4.06d	10.20g	3.67f	9.83g
Untreated Control	9.70c	17.96d	9.70b	17.96c	9.70b	17.96c	9.70a	17.97b	9.70a	17.97b
L.S.D. at 0.05	0.66	1.62	0.49	0.56	0.48	0.69	0.37	0.63	0.39	0.74

- Mean values in each column followed by the same letter are not significantly different (P ≤ 0.05).

Effect of bioagents on the root and shoot dry weight of pepper plants infected with root-rot pathogens

Results in Table 6 revealed that the tested bioagents significantly enhanced the roots and shoots dry weight of pepper plants infected with *P. aphanidermatum, F. solani, F. oxysporum, F. moniliformis* and *M. phaseolina* compared to the infected control treatment. Interestingly, the TV1 bioagent promoted the dry weight of pepper roots and shoots to the maximum extent that were infected with all five tested pathogens compared to other bioagents or the positive fungicide. The bioagents directly attack the plant pathogens by secreting lytic enzymes including chitinases, these enzymes hydrolysis the pathogen cell wall as chitin in principal and other components as glycan causing dramatic effect on pathogenic fungi (Chandanie et al., 2009; Hermosa et al., 2013).

TABLE 6. Effect of bioagents on the root and shoot dry weight of pepper plants infected with root-rot pathogens

Treatment	Pathogens									
	Pythium aphanidermatum		***Fusarium solani***		***Fusarium oxysporum***		***Fusarium moniliformis***		***Macrophomina phaseolina***	
	Root	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**
T. harzianum (TH2)	1.97b	8.00a	1.77b	6.40cd	1.83bc	8.12 b	1.67b	5.97d	2.47b	6.17c
T. viride (TV1)	2.2a	7.07a	2.40a	9.73a	2.47 a	9.40 a	2.43a	8.10a	2.70a	9.77a
P. fluorescens (P2)	1.62cd	5.50b	1.60bc	5.83cd	1.70 bc	5.11 c	2.37a	6.67bc	2.00c	6.07c
B. subtilis (B1)	1.7c	5.50b	1.90 b	7.00bc	2.00 b	5.83 c	1.83b	6.27cd	2.47b	8.67b
Hatric	1.63cd	5.10b	1.70bc	6.50cd	1.67 c	5.84 c	1.80b	7.10b	1.77d	6.23c
Infected Control	1.38d	3.76c	1.33 c	4.46e	1.23 d	3.87 d	1.40c	4.00e	1.43e	2.77d
Untreated Control	1.84bc	7.67a	1.84b	7.67b	1.84bc	7.67 b	1.84b	7.67a	1.84cd	7.67b
L.S.D. at 0.05	0.21	0.77	0.31	0.83	0.22	0.64	0.17	0.55	0.14	0.70

- Mean values in each column followed by the same letter are not significantly different (P ≤ .05).

According to the results of this study, biological control could be considered the best alternative and may be helpful, especially against soil borne pathogens. Obtained results match with those obtained by (Pandya and Saraf, 2010) who mentioned that biological control is a well-established fact where antagonistic fungi play an important part of biological control that gained wide acceptance next to *Bacillus thuringiensis* and *P. fluorescens* because of their broader spectrum in terms of disease control and yield.

Conclusion

Bioagents isolated from rhizospher of healthy pepper pants may be effective against the pepper root rot pathogens and could have a potential as biofertilizer effect, since they stimulated growth of pepper plants compared to control.

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